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DR. Food ™ Kit User's Guide

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DR. Food ™ Kit *User's Guide*

Notes

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1.Kit Contents

Specifications

Part A: Storage at –20°C or below

No	Contents	Volume	Quantity
1	PC	300 µL	1 Vial
2	DNA Pol.	55 µL	1 vial
3	FM	1.3 mL	4 vials

Part B: Storage at 2-8°C

No	Descriptions	Volume	Quantity
1	DR. Hyb™ Buffer	60 mL	1 bottle
2	Wash Buffer	200 mL	2 bottles
3	Strep-AP	55 µL	1 vial
4	Blocking Reagent	60 mL	1 bottle
5	Detection Buffer	120 mL	1 bottle
6	NBT/BCIP	1.2 mL	1 vial
7	E1 (10X, concentrate)	20 mL	1 bottle
8	E2	1.3 mL	4 vials
9	DR. Food Chip	3 reactions	32 packs

Description of products

No	Reagent	Description
1	**PC**	Positive control DNA template of PCR
2	**DNA Pol.**	PCR enzyme (5U/µL).

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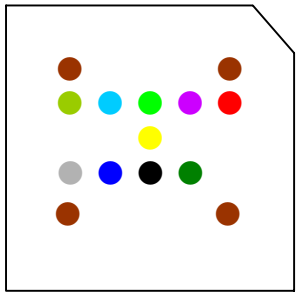
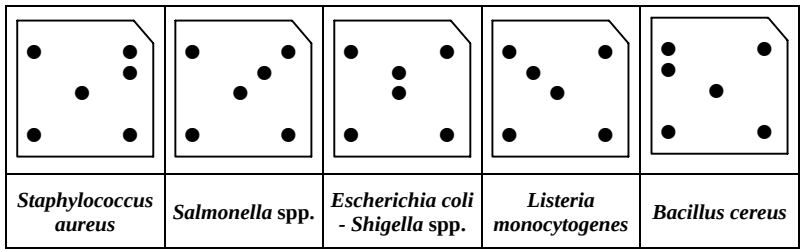
Notes

Allocation of specific oligonucleotide probes

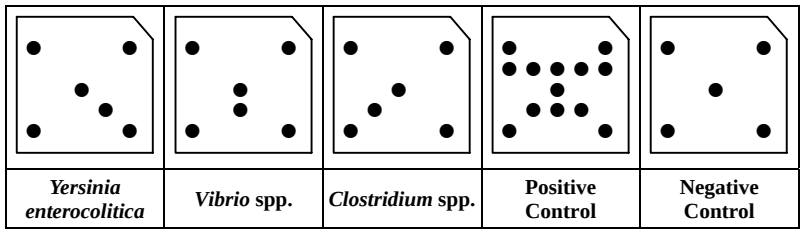


- Hybridization Positive Control
- *Staphylococcus aureus*
- *Salmonella* spp.
- *Escherichia coli* - *Shigella* spp.
- *Listeria monocytogenes*
- *Bacillus cereus*
- *Yersinia enterocolitica*
- *Vibrio* spp.
- *Clostridium* spp.
- PCR positive control
- Negative control

Interpretation of results



Staphylococcus aureus	***Salmonella* spp.**	***Escherichia coli* - *Shigella* spp.**	***Listeria monocytogenes***	***Bacillus cereus***



Yersinia enterocolitica	***Vibrio* spp.**	***Clostridium* spp.**	**Positive Control**	**Negative Control**

3	**FM**	PCR premix for amplifying specific target genes: *Staphylococcus aureus*, *Escherichia coli*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., and DNA internal control; the reagent contains specific 5'-biotinylated primer pairs, dNTPs, PCR buffer, and $MgCl_2$.
4	**DR. Hyb™ Buffer**	Specially formulated to facilitate hybridization of specific oligo probes with target DNA amplicons.
5	**Wash Buffer**	Stringency wash to remove non-binding amplicons, after hybridization reaction.
6	**Strep-AP**	Using the specificity of biotin and streptavidin to detect the biotinylated molecules of amplicons. The streptavidin conjugated alkaline phosphatase as enzyme to react with its substrate NBT/BCIP for colorimetric signal on the chip.
7	**Blocking Reagent**	Specifically formulated to reduce non-specific nucleic acid binding on polymer chip surfaces, also as a dilution buffer for Strep-AP.
8	**Detection Buffer**	A dilution buffer for NBT/BCIP, formulated to ensure Strep-AP activity.
9	**NBT/BCIP**	Chromogenic phosphatase substrates. Hydrolysis of BCIP (5-Bromo-4-chloro-3-indolyl phosphate indolyl phosphate), followed by oxidation, produces a blue-colored precipitate at the site of enzymatic activity. NBT (Nitro blue tetrazolium) is the most commonly used electron-transfer agent and co-precipitant for the BCIP reaction, forming a dark blue, precisely localized precipitate in the presence of Strep-AP.
10	**E1**	Remove the PCR inhibitor for bacterial culture medium.

11	**E2**	Extract genomic DNA from bacteria
12	**DR. Food™ Chip**	A platform for hybridization reaction. Its surface is pre-spotted with a variety of specific probes for target organisms.

Storage conditions

Please store **Part A** at -20°C or below, **Part B** at 2-8°C upon delivery. DR. Food™ Kit has demonstrated stability over a 9 month period when stored at recommended temperatures; however, we recommend to be discard the kit after expiration date.

Precaution

- Aliquot **FM**, **DNA Pol.**, **PC** prior to use.
- Do NOT leave **DNA Pol**. at room temperature over 5 minutes.
- **Only appropriately trained molecular laboratory personnel shall operate this system. All protocols in this manual shall be followed. Gloves shall be worn at all time. In case of contact with eyes, rinse eyes immediately with plenty of water.**
- **Do NOT touch the surface of the chip.**
- **Before using E1, add 180 mL sterile water to obtain 200 mL**

2. Mix 10 μL NBT/BCIP with 490 μL Detection Buffer for each chi**p,** transfer the mixture to the hybridization chamber.
3. Allow to react in the dark for 10 minutes at room temperature.
4. Discard the detection liquid, rinse the chip with 1 mL water twice and read the results of the pattern developed on the chip with DR. AiM Reader.

4. Results

Pattern design principle

Results are interpreted by the pattern formed on the chip. Foodborne pathogen-specific oligonucleotide probes are designed and pre-spotted on the chip to capture specific target amplicons. After hybridization and colorimetric development, perfectly matched probe-target hybrid will form a blue-purple precipitate on the chip as a result of enzymatic reaction. Chip patterns are read and identified by DR. AiM™ reader together with analysis software (Refer to the DR. AiM™ Reader user's guide). Diagrams below show the patterns of the DR. Food™ kit.

Note: A successful test will show the relevant spots for Hybridization Positive Control and PCR Internal positive Control. In the absence of the positive controls, please check the operation process carefully.

V. Hybridization

Preparation for hybridization

1. Pre-warm DR. Hyb Buffer, Wash Buffer and chip to room temperature.
2. Pre-heat hybridization oven to 45 °C.

Hybridization Reaction

3. Pipette 25 µL amplicons into 500 µL pre-warmed DR. Hyb Buffer and mix thoroughly.
4. Denature mixture in boiling water for 10 minutes.
5. Chill on ice for 3 minutes immediately, and then spin down.
6. Transfer 500µL of hybridization mixture onto the DR. Food™ Chip; incubate at 45 °C in the DR. Mini Oven with maximum vibration for 1 hour.

VI. Stringency Wash

1. Discard the hybridization liquid.
2. Add 500µL Wash Buffer into the hybridization chamber and then discard the wash liquid. Repeat this wash step three or four times.
3. After step 2, invert the chip chamber and repeatedly tap on paper towel to remove any residual liquid.

VII. Colorimetric Development

Blocking

1 For each chip mix 0.5 µL Strept-AP with 500 µL Blocking Reagent; transfer the mixture into the chamber, allow to react for 30 minutes at 25~35 °C.
2. Discard the reacted blocking liquid.
3. Add 500µL Wash Buffer into the hybridization chamber and then discard the wash liquid. Repeat this wash step three or four times.
4. After step 3, invert the chip chamber and repeatedly tap on paper towel to remove any residual liquid.

Colorimetric Reaction

1. Rinse chip with 500 µL Detection Buffer and discard the liquid.

2.Introduction

Product Characteristics

DR. Food™ kit is a foodborne pathogen detection system for *Staphylococcus aureus*, *Escherichia coli*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium spp.* (*Clo. perfringens and Clo. butulinum*), *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., and *Vibrio* spp.. The system provides more accurate test results than bacterial culture. DR. Food™ kit system applies four molecular biology techniques: DNA extraction, PCR amplification, DNA hybridization and colorimetric development, using the following principle:
The genomic DNA of the pathogens is extracted from bacterial enrichment culture and the target gene is replicated using PCR amplification technology. The amplicons are hybridized with specific probes, which are found on the chip. After hybridization, stringent washing removes non-target DNA fragments and residual amplicons. After subsequent colorimetric development, patterns will form on the chip corresponded to the pathogen within the food sample.

Limitations

DR. Food™ kit is designed to detect nucleic acids of the foodborne pathogens, *Staphylococcus aureus*, *Escherichia coli*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium spp.* (*Clo. perfringens and Clo. butulinum*), *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., and *Vibrio* spp.. The detection limit is listed below for bacteria after enrichment.

Pathogen	Sensitivity (cfu/mL)	Pathogen	Sensitivity (cfu/mL)
Staphylococcus aureus	$10^9 \sim 10^4$	*Salmonella* spp.	$10^9 \sim 10^4$
Escherichia coli	$10^8 \sim 10^4$	*Shigella* spp.	$10^8 \sim 10^4$
Yersinia enterocolitica	$10^8 \sim 10^4$	*Vibrio* spp.	$10^8 \sim 10^4$
Bacillus cereus	$10^8 \sim 10^3$	*Clostridium* spp.	$10^8 \sim 10^3$
Listeria monocytogenes	$10^9 \sim 10^4$		

3.Procedure and materials required

Materials required but not provided

Instruments

- Micropipettes
- Vortex mixer
- -20℃ Freezer
- Hot plate
- DR. AiM™ Reader
- DR. Mini Oven
- Microcentrifuge
- Thermal cycler (Suggestion : PTC-100/MJ Research ,Inc.)

Consumables

- PCR tubes
- Tips
- 1.5 mL microcentrifuge tubes

Procedure

I. Sampling & Preparation

Sampling methods and preparation vary depending upon the type of food. Check national regulations for detailed methods of food sample preparation or use of the methods described in Food Sampling and Preparation of Sample Homogenate (Bacterial Analytical Manual Online, Chapter 1, http://www.cfsan.fda.gov/~ebam/bam-1.html) by FDA as reference.

II. Enrichment

1. Add 25 mL or 25 g food sample in 225 mL LB broth (or other suitable medium), incubate at 37℃, 24 +/- 2 hours)
2. Carry out ten-fold dilution of this enrichment culture with sterile water before DNA extraction step.

III. DNA Extraction

- **Before using E1, add 180 mL sterile water to obtain 200 mL**

1. Transfer 1 mL of diluent, which was obtained from previous step, into a microcentrifuge tube and centrifuge at 8,000 g/ 4℃ for 5 minutes.
2. Remove the supernatant, resuspend pellet with 1 ml E1 and mix gently.
3. Centrifuge at 8,000 g/ 4 ℃ for 5 minutes

4. Discard the supernatant, and resuspend the pellet with 50 µL E2; vortex until mixed well.
5. Heat in boiling water for 10 minutes; chill on ice for 2 minutes
6. Vortex the mixture and centrifuge at 8,000 g/ 4℃ for 5 minutes.
7. Take 50 µL supernatant, containing the isolated DNA, to a new 1.5 ml eppendorf tube ready for amplification. The extracted DNA can be stored at 4°C and used within 2 days.

IV. DNA Amplification

1. *Preparation of PCR Master Mix.* Transfer the following reagents into a PCR tube and mix thoroughly. For every batch of tests, prepare additional tubes for PCR negative control and positive control.

Reagents	Volume/tube	# of tubes	Total volume
FM	39.5 µL	X (n + 1)	= _____µL
DNA Pol.	0.5 µL	X (n + 1)	= _____µL

✪ n = number of sample (including PCR negative control and positive control)

2. Pipette 40 µL master mix into each PCR tube.
3. Transfer 10 µL extracted DNA sample into each tube, mix thoroughly; for PCR negative control, use 10 µL aseptic water and for PCR positive control, use 10 µL PC (positive control template) reagent. Final volume of each tube is 50 µL.
4. Place all PCR tubes in a Thermal Cycler and follow the amplification program listed below.

95 °C	5 min	
95 °C	30 sec	30 cycles
60 °C	30 sec	
72 °C	30 sec	
72 °C	5 min	
4 °C	~	

5. Proceed to hybridization reaction.

✪ If not used immediately, please store amplicons at -20 °C